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# Protein Intakes and Growth of Breastfed and Breastfed Supplemented Infants During the First Six Months of Age

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## ABSTRACT

Protein concentration in human milk from 39 well-nourished American women and its adequacy for growth of exclusively breastfed infants(BF) and breastfed infants fed supplementary foods(BFS) from 1 - 6 months postpartum were studied.

Mean protein concentration of breast milk measured by Lowry et al., using human serum albumin as a standard, over the first 6 months lactation was 1.31±0.13g/dl. Concentration of protein was significantly higher at the first month of lactation(1.55±0.23g/dl)($P<0.05$) than any other month studied. Mean volume of breast milk ranged from 662 - 848ml/day in the BF group and from 415 - 661ml/day in the BFS group during the first 6 months of lactation. Mean protein intake of infants ranged from 1.3 - 2.2g/kg in the BF group and from 1.4 - 2.1g/kg in the BFS group.

Mean protein intake(g/kg body weight) of both BF and BFS groups was less than Recommended Dietary Allowance(1989, USA) 0f 2.2g/kg except at 1 month of age. However, mean growth of the infants was normal according to NCHS reference, suggesting that the RDA for protein was unrealistically high for infants during 2 - 6 months of age. Protein provided by breast milk alone appeared adequate for normal growth during this time. (*Korean J Nutrition* 29(8) : 908~915, 1996)

**KEY WORDS** : breastfed · human milk · protein intake · infant growth · RDA(1989, USA).

## Introduction

Advantages of breastfeeding are well recognized as evidenced by several recent position papers by national and international organizations[1-6]. Answers to the questions "when does breast milk alone become nutritionally inadequate for the infant?" and "when are supplementary foods necessary?" require knowledge of the compositon of breast milk as well as the nutrient requirements of infants. These questions are remarkably difficult to answer insofar as the protein needs of individual infants are concerned.

Current protein allowances(RDA, 1989, USA) have been based largely on observations of thriving infants[7]. However, a discrepancy is evident between the volume of human milk required to meet the protein allowances of infants and the usual volume of human milk production[8,9]. The recommended protein allowances(RDA, 1989, USA) for infants ranging from 0 - 0.5 yr and 0.5 - 1 yr of age are 2.2 and 1.6g/kg body weight, respectively. The amount of human milk necessary to meet these allowances would require yields in excess of the reported yields of 600 to 900ml/day[10-14].

Our study was designed, 1) to document the pro-

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tein intakes and growth of exclusively breastfed infants(BF) and of breastfed infants fed supplementary foods(BFS) during the first 6 months of age in order to obtain normative data of protein intakes of these two groups of infants and 2) to examine the relationship between the observed levels of protein intakes of these two groups and the current recommended dietary allowance of protein for infants (RDA, 1989 USA).

## Materials and methods

### 1. Subject description

The study population consisted of 39 American pregnant women who lived in a Purdue University (West Lafayette, Indiana, USA) community. Subjects were recruited into the study a few weeks before their deliveries. All procedures used in the study were approved by the Purdue University Committee on the use of Human Subjects in research. Mothers with drinking alcohol, using drugs and having smoking habit were excluded. Mean age of these mothers was 29(ranged 22 - 37)yrs, mean weight was 59 kg(ranged 44 - 101) and mean BMI was 21.7(ranged 16.7 - 36.7) $kg/m^2$. The mothers were in apparent good health and without any clinial evidence of disease.

### 2. Breast milk collection and dietary information

Subjects were requested to collect a sample of milk at each feeding during a 24hr period, monthly from 1 to 6 months postpartum. Samples of approximately 10ml of milk were expressed after milk let-down either manually or by the use of an acid-washed breast pump(Loyd-B-Pump, Lopuco Ltd., Woodbine, MD) into amber-colored plastic vials. All samples were stored immediately in the subjects' home freezers and were transferred within 2 days to the laboratory where they were stored at -30 ℃ until analysis. On the day of milk sampling the mothers were asked to keep a detailed 24hr record of their food intake and that of their infants when food other than breast milk consumed.

### 3. Sample analysis

Total protein content of breast milk samples was determined by the method of Lowry et al. using human serum albumin as a standard[15]. Protein content of supple-mentary foods were estimated by use of food composition data from the Composition of Foods, Agricultural Handbook No. 8-3 Science and Education Administration, revised 1978.

### 4. Volume of breast milk intake and pro-tein intake

Volume of breast milk was estimated by use of a test weighing procedure at each feeding during a 24hr period. Mothers were instructed the use of a K-tron portable electronic balance(7955 East Redfield Road, Scottsdale, AZ), accurate to 1g, which was used for weighing the infants. Differences in infant weights before and after a feeding without a change in clothing were recorded in grams and were then converted to volume by use of the mean reported specific gravity of human milk(1.031)[16].

A subset of seven exclusively breastfed infants(BF-e) were followed individually throughout the 6-month study for comparison with the BF group which did not include the same individuals each month.

Protein intake from breast milk was calculated by multiplying volume of breast milk intake at each feeding by the protein concentration analyzed in milk samples collected at each feeding. And Protein intake from supplementary foods in BFS was calculated by dietary information from mothers. These values were summed to obtain protein intakes of each infant during a 24hr period.

### 5. Assessment of infant growth

Length and weight measurements of infants were obtained each month during the 6 month study by one trained technician. Weights were measured by the use of a K-tron eletronic balance and lengths were measured by the use of a portable tape board equipped with a head and foot board. These measurements were compared with growth reference percentiles published by the the National Center for Health Statistics(NCHS)[17].

### 6. Statistical analysis

Analyses were carried out the use of the Statistical Package for Social Science(SPSS)[18]. Mean volume of milk intake, measured by test weighing, and protein

intake were analyzed by a one-way analysis of variance. The Newman-Keuls sequential range test was used to determine significant differences($P<0.05$) among means of different months. Regression analysis of protein concentration in milk and volume of milk intake was used to determine Pearson correlation coefficients.

## Results

### 1. Protein concentration

Mean protein concentration of breast milk over the first 6 months lactation was 1.31±0.13g/dl. Concentration of protein was significantly higher at the first month of lactation(1.55±0.23g/dl)($P<0.05$) than at any other month studied. From 2 to 6 months, mean protein concentration was not significantly different (Table 1). Protein concentrations measured at different feeding time during a day did not vary significantly during the first 6 months of lactation.

**Table 1.** Protein concentration of breast milk at different stages of lactation from 1 to 6 months

| Stage of Lactation (Month) | Number of Mothers | Protein Concentration (g/dl) |
|---|---|---|
| 1 | 38 | 1.55±0.04[1)]* (1.2 – 2.3)[2)] |
| 2 | 39 | 1.35±0.03 (1.0 – 1.9) |
| 3 | 39 | 1.26±0.03 (0.9 – 1.6) |
| 4 | 39 | 1.24±0.03 (1.0 – 1.6) |
| 5 | 37 | 1.22±0.03 (0.8 – 1.7) |
| 6 | 37 | 1.25±0.03 (0.9 – 1.7) |
| Total | | 1.31±0.13 (0.8 – 2.3) |

1) Mean±SE
2) Range.
*Mean significantly different($P<0.05$) from means of all other months.

**Table 2.** Volume of breast milk intake and protein intake of exclusively breastfed infants(BF) and breast fed infants fed supplementary foods(BFS) during the first 6 months of age

| Treatment Group | Age of Infant(Month) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | Number of Infants | | | | | |
| BF | 36 | 36 | 34 | 25 | 19 | 7 |
| BFS | 2 | 3 | 5 | 14 | 18 | 30 |
| Breast milk Intake | ml/day | | | | | |
| BF | 690[1)A] | 682[A] | 662[A] | 698[A] | 750*[A] | 848*[A] |
| | (159)[2)] | (161) | (143) | (179) | (147) | (239) |
| BFS | 415[A] | 600[A] | 592[A] | 661[A] | 577[A] | 562[A] |
| | (114) | (192) | (202) | (196) | (159) | (195) |
| | ml/kg/day | | | | | |
| BF | 144[A] | 119[B] | 103[B] | 102[B] | 103*[B] | 110*[B] |
| | (31) | (26) | (22) | (26) | (18) | (36) |
| BFS | 94[A] | 99[A] | 91[A] | 89[A] | 76[A] | 71[A] |
| | (22) | (18) | (31) | (22) | (20) | (24) |
| Protein Intake | g/day | | | | | |
| BF | 10.7[A] | 9.1[B] | 8.3*[B] | 8.7*[B] | 9.5[AB] | 10.5[AB] |
| | (2.6) | (2.1) | (2.0) | (2.3) | (2.2) | (2.6) |
| BFS | 9.4[A] | 12.1[A] | 10.9[A] | 10.5[A] | 10.6[A] | 11.9[A] |
| | (2.4) | (2.2) | (3.7) | (3.1) | (2.8) | (5.7) |
| | g/kg/day | | | | | |
| BF | 2.2[A] | 1.6[B] | 1.3*[C] | 1.3[C] | 1.3[C] | 1.4[BC] |
| | (0.6) | (0.4) | (0.3) | (0.3) | (0.3) | (0.4) |
| BFS | 2.1[AB] | 2.1[A] | 1.7[B] | 1.4[B] | 1.4[B] | 1.5[B] |
| | (0.6) | (0.7) | (0.6) | (0.4) | (0.4) | (0.6) |

1) Mean
2) (SD)
*Mean significantly different($P<0.05$) from means of BFS group
A, B, C means within a row without a common superscript letter are significantly different from each other($P<0.05$)

### 2. Volume of breast milk intake and pro-tein intake

Mean volume of breast milk intake(ml/day and ml/kg body weight/day) and protein intake(g/day and g/kg body weight/day) from 1 to 6 months are shown for breastfed infants(BF) and breastfed infants receiving supplementary foods(BFS) in Table 2.

During the 6-month study, breast milk intake was not significantly different for the BF and BF-e groups (Fig. 1). Breast milk intake was significantly higher in BF group than in the BFS group at 5 and 6 months when a large number of infants were fed supplements (Table 2).

Supplementary foods were fed to 36% of the infants at 4 months and 81% at 6 months of age. The pattern of milk intake/kg body weight of both BF and BFS groups during the first 6 months of age(Fig. 1) paralleled the patterns of weight gain.

Protein intake(g/day, g/kg body weight/day) of BF and BF-e groups were not significantly different during the 6-month study(Fig. 2). Protein intake was significantly higher for the BFS than the BF group at 3 and 4 months but not at 5 and 6 months when the volume of milk intake of the BF group was significantly higher than BFS.

Protein intake/kg body weight/day was significantly higher for the BFS group only at 3 months (Table 2). The pattern of protein intake(g/kg/day) is shown in Fig 2 : for all groups, protein intake calculated on the basis of body weight declined rapidly during the 3 months of the study.

### 3. Factors related to protein intake

Protein intakes of infants were significantly correlated with the volume of breast milk intake throughout the first 6 months of age. Pearson r values ranged from 0.81 at 1 month to 0.91 at 6 months($p<0.01$)(Fig. 3). Values for 2, 3, 4 and 5 months were 0.78, 0.81, 0.85, and 0.78, respectively. Protein concentration of milk was not significantly correlated with protein intakes of infants except at 1 month of age($r=0.43$, $p<0.01$).

### 4. Supplementary foods intake

Protein intake from supplementaty foods during the

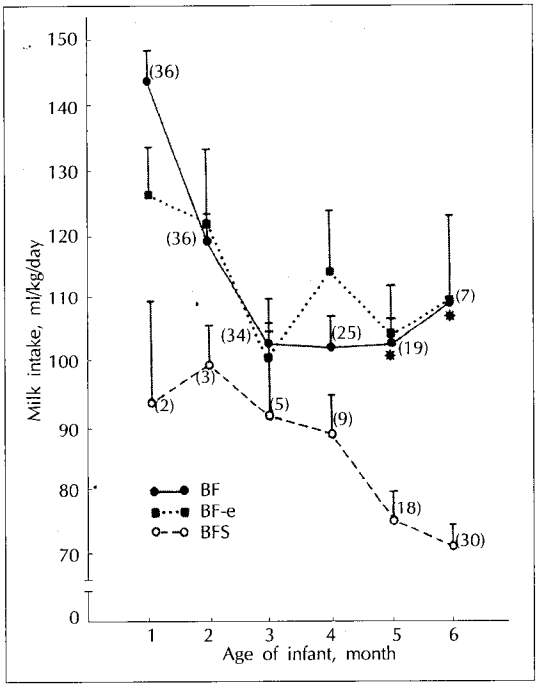


**Fig. 1.** *Volume of milk intake(ml/kg/d)of exclusively breastfed infants(BF) and those fed supplementary foods (BFS). In addition, data are presented for 7 infants who were BF throughout the first 6 mo(BF-e). Vertical bars represent mean±SEM. *Means significantly different($P<0.05$) from means of BFS.*

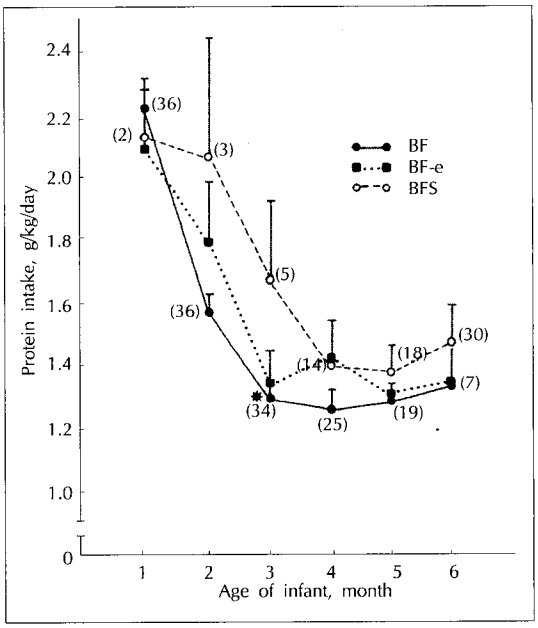


**Fig. 2.** Protein intake(g/kg/d) of exclusively breastfed infants(BF) and those fed supplementary foods(BFS). In addition, data are presented for 7 infants who were BF throughout the first 6 mo(BF-e). Vertical bars represent mean±SEM. *Means significantly different($P<0.05$) from means of BFS.

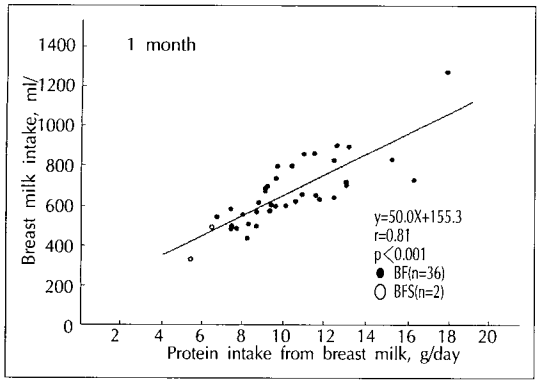


**Fig. 3.** Correlation between volume of breast milk intake and protein intake from breast milk of infants at 1 month of age.

first 6 months of age was observed. At 1 to 3 months, formula was the chief supplementary food(94–100%). Thereafter other supplementary foods were introduced and the percentage of formula intake relative to other supplementary foods was less after 3 months. At 5 months, almost 50% of the infants and at 6 months, 81% of the infants received supplementary foods contained protein. Still at 6 months of age 7 infants(20%) did not receive any supplementary foods.

### 5. Infant growth

Mean weight, length, and weight gain of BF and BFS during 6-month study period are shown in Table 3. Body weights and lengths of the BF and BFS groups were not significantly different except at 4 months of age. Weight gains declined in both groups during the study peroid but were not significantly different between the two groups(Table 3). Growth performance of the BF infants at 1 and 3 months and of BFS infants at 6 months were evaluated by examining the frequency distribution of the percentile categories of weight-for-age and length-for-age according to the NCHS reference[17]. Weight-for-age and length-for-age of more than 70% of the infants in the BF and BFS groups exceeded the 50th percentile during the first 6 months.

## Discussion

Mean volume of breast milk intake of approximately 700ml/day by infants in this study was consistent with previous reports in the literature[10-14]. Furthermore no significant variation in milk intakes was observed over the first six months in either the exclusively breastfed or the breastfed infants fed supplementary foods. Breast milk production was probably related to mental stress in one mother, resulting in a low volume of milk production at 1 month (335ml/day) ; after the stress lessened milk production became normal. The infant of this mother gained significantly less body weight than any other infant during the first month(11.4g/day compared to a

**Table 3.** Body weights and lengths of exclusively breastfed infants(BF) and breastfed infants fed supplementary foods(BFS) during the first 6 months of age

| Treatment Group | Age of Infant(Month) | Body Weight (kg) | Length (cm) | Weight Gain (% increase) |
|---|---|---|---|---|
| BF (36)[1)] | 1 | 4.8 ± 0.6[2)A] | 56.7 ± 3.0[A] | 30.6 ± 5.2 |
| BFS (2) | | 4.4 ± 0.2 | 55.3 ± 2.7 | 18.1 ± 8.1 |
| BF (36) | 2 | 5.7 ± 0.6[B] | 60.2 ± 2.8[B] | 18.8 ± 7.6 |
| BFS (3) | | 6.0 ± 0.8 | 62.5 ± 2.6 | 36.4 ± 6.9 |
| BF (34) | 3 | 6.5 ± 0.8[C] | 62.8 ± 3.4[C] | 14.0 ± 6.5 |
| BFS (5) | | 6.5 ± 0.3 | 64.8 ± 2.5 | 8.3 ± 5.5 |
| BF (25) | 4 | 6.8 ± 0.6*[D] | 64.8 ± 2.3*[D] | 4.6 ± 2.8 |
| BFS (14) | | 7.4 ± 0.8 | 66.8 ± 3.0 | 13.9 ± 7.8 |
| BF (19) | 5 | 7.3 ± 0.7[E] | 66.6 ± 2.6[E] | 7.4 ± 3.5 |
| BFS (18) | | 7.6 ± 0.8 | 68.4 ± 3.6 | 2.7 ± 1.1 |
| BF (7) | 6 | 7.6 ± 0.7[E] | 69.0 ± 3.2[E] | 4.1 ± 2.4 |
| BFS (30) | | 7.9 ± 0.8 | 69.6 ± 3.7 | 3.9 ± 1.2 |

1) Number of infants
2) Mean ± SD
*Mean Significantly different($P<0.05$) from mean of the BFS group
A, B, C, D, E Means within a column for BF group without a common superscript letter are significantly different from each other($P<0.05$)

mean of 37.5g/day for other infants) even though supplementary food was given to the infant during this time.

Mean protein intake of the BF group was higher than some reported values[9)19)], but was in agreement the estimated protein requirements of breastfed infants recommended by Butte and Garza[20)]. Even though protein intake(g/day) was significantly higher in the BFS group than the BF group at 3 and 4 months, body weights of these two groups of infants similar during the 6-month study. The data indicated that during the first 6 months, BF infants increased their volume of milk intake resulting in protein intakes similar to the BFS group. Approximately 70% of BF group exceeded the 50th percentile of weight-for-age and 60% exceeded the 50th percentile of length-for-age. This suggested that breastfeeding alone provided adequate protein for growth and that protein from supplementary foods was not essential to normal growth during the first 6 months of age. The volume of breast milk, rather than its protein concentration, was associated with protein intake and growth of infants during the first 6 months of age.

Protein requirements per kg of body weight of infants would be expected to decrease during the first 6 months of age parallel to the deceleration in growth velocity especially during the first month when growth decelerates most rapidly. In this study both weight gain and protein intakes of infants at 1 month of age were significantly higher than any other age studied. For the first month of age, estimated requirements are based on intake data because of the difficulty in accurately estimating allowances for growth and maturation of body composition. According to a WHO report[21)] protein intakes by breastfed infants ranged from 2.43g/kg/day in the first month to 1.51g/kg/day in the fourth month and averaged 2.04g/kg/day in the first 3 months and 1.73g/kg/day in the next 3 months. Butte et al.[9)] suggested that breastfed infants in the United States grow satisfactorily when mean protein intake is 1.68g/kg/day(total protein $\times 6.25$) during the first 3 months. Beaton and Cherry[22)] concluded from the probability assessment analyses that the actual mean protein requirement of breastfed infants ages 3 to 4 months of age was 1.1g/kg/day.

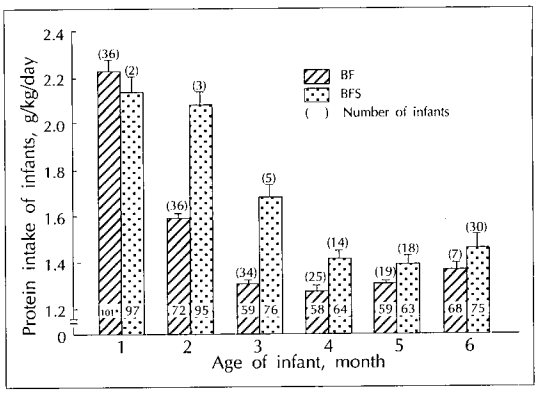


**Fig. 4.** Protein intakes of exclusively breastfed infants(BF) and those fed supplementary foods(BFS) during the first 6 months of age. Vertical bars represent mean ±SEM. *Percent Recommended Dietary Allowance (1989).

In the present study protein intakes of both BF and BFS groups were considerably lower than the recommended intake(RDA, 1989, USA)(Fig. 4) except at 1 month of age, even though growth was apparently normal according to NCHS percentile. The data suggested that solely breastfed infants probably consumed adequate amounts of protein for growth during the first 6 months. The recent recommended dietary allowances for protein intake of infants(RDA, 1989, USA) appear to be high for infants 4 to 6 months of age. The data from this study indicated that protein provided by breast milk alone was adequate for normal growth during the first 6 months.

## Summary and Conclusion

Protein concentration in breast milk of healthy, well-nourished American women was examined for its adequacy for growth of exclusively breastfed infants(BF) during the first 6 months of age. These data were compared to that of breastfed infants fed supplementary foods(BFS).

Mean protein concentration of breast milk ranged from 1.2 to 1.6g/dl during the first 6 months of lactation and was significantly higher during the first month of lactation than any other month studied. Protein concentrations did not vary significantly at different feedings during a 24hr period.

Mean volume of breast milk intake at each monthly sampling during the first 6 months BFS group. Volume of breast milk intakes(ml/day and ml/kg

body weight/day) of the BF group and an group of 7 infants exclusively breastfed for 6 months(BF-e) were not significantly different. Milk intakes of these groups were significantly higher than that of the BFS group at 5 and 6 months of age. Mean protein intake of infants at each monthly sampling during the first 6 months ranged from 1.3－2.2g/kg in the BF group and from 1.4－2.1g/kg in the BFS group. Protein intake of the BF and BF-e groups were not significantly different during the first 6 months, however, that of BFS group was significantly higher than the BF group at 2－4 months of age. Protein intake of the BFS calculated on the basis of per kg body weight was significantly higher than of the BF group at 2 and 3 months of age.

The data showed that BFS infants decreased the volume of milk intake to compensate for the protein received from supplementary foods during 4 to 6 months.

Protein intake was strongly correlated with volume of breast milk intake during the first 6 months of age. Protein concentration of breast milk was not significantly correlated with protein intake of infants except at 1 month when the concentration was highest.

Mean protein intake per kg body weight of both BF and BFS groups was less than Recommended Dietary Allowance(1989, USA) 0f 2.2g/kg except at 1 month of age. However, mean growth of the infants was normal according to NCHS reference, suggesting that the RDA for protein was unrealistically high for infants during 2－6 months of age. Protein provided by breast milk alone appeared adequate for normal growth during this time.

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= 국문초록 =

# 생후 6개월 동안 모유영양아의 단백질 섭취량과 성장과의 관계

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이 연구는 영양상태가 좋은 39명의 미국여성들의 모유내 단백질 농도를 분석하고 그것이 생후 6개월 동안 모유영양만 섭취한 군(BF)과 모유와 보충식이를 섭취한 군(BFS)의 성장에 적합한가를 알아보기 위해 연구 되었다.

처음 6개월간의 모유내 단백질 농도는 혈청 알부민(Human)을 표준으로 사용한 Lowry등의 방법에 의해 분석되었으며 그 평균 값은 1.31±0.13g/dl 이었다. 이 값은 첫번째 달에서 다른달에 비해 모유내 단백질 농도가 유의적으로 높았다(1.55±0.23g/dl). 모유는 평균적으로 BF군에서는 662-848ml/day BFS 군에서는 415-551ml/day로 섭취 되었으며 이 값은 첫번째 달을 제외하고는 두군에 있어서 유의적인 차이는 보이지 않았다. 영아의 평균 단백질 섭취량은 BF군에서 1.3~2.2g/kg BFS군에서는 1.4~2.1g/kg 이었으며 이 값은 6개월 내내 두군에 있어서 유의적인 차이를 보이지 않았다.

두군의 평균 단백질 섭취량(g/kg)은 첫번째 달을 제외하고는 현 미국 RDA인 2.2g/kg보다 적은 량이다. 그러나 NCHS 기준에 의하면 이 영아들의 성장은 지극히 정상이며 생후 6개월간은 영아의 정상적인 성장이 가장 중요한 영양상태의 지표임을 고려하면 단백질에 관한 이 RDA 값은 2~6개월 동안의 영아들에는 비현실적으로 높은것이다. 또한 모유만에 의해서 제공되는 단백질은 이 기간동안 정상적인 성장에 적당한 것으로 생각된다.